

# Electron Microscopic Findings of Cells with Inclusion Bodies in Experimental Hemorrhagic Enteritis of Turkeys

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## ABSTRACT

The spleen, liver, bone marrow and intestines of two turkeys in which hemorrhagic enteritis of turkeys was experimentally reproduced were examined electron-microscopically. Intranuclear inclusion bodies as described in a previous report were found in all the tissues examined. These occupied most of the area in affected nuclei and were composed of viral particles with morphological characteristics of an adenovirus. The cells with the inclusions were divided into two types of cells, immature and reticular cells. There was some variety in the stage of differentiation of the former cells. As the viral particles developed the cells degenerated and disintegrated. A few particles had been released into the cytoplasm of the degenerated cells but no particles were present in intercellular spaces.

## RÉSUMÉ

Les auteurs ont examiné au microscope électronique des coupes de la rate, du foie, de la moelle osseuse et des intestins de deux dindes atteints d'entérite hémorragique expérimentale.

Ils décelèrent des corps d'inclusion identiques à ceux qu'il ont décrits dans un autre article, au sein de tous les tissus qu'ils examinèrent. Ces inclusions remplissaient pratiquement les noyaux lésés et se composaient de particules virales possédant les caractères morphologiques d'un adénovirus. Les corps d'inclusion se retrouvaient dans les cellules réticulées ou dans des cellules immatures n'ayant pas toutes atteint le même stade de différenciation. À mesure que les particules virales se développaient, les cellules subissaient la dégénérescence et la désintégration. Quelques particules virales s'étaient échappées dans le cytoplasme des cellules dégénérées, mais on n'en retrouva pas dans les espaces inter-cellulaires.

## INTRODUCTION

Recently it has been shown that formation of intranuclear inclusion bodies is a histological lesion characteristic of hemorrhagic enteritis of turkeys (3, 5). In addition, viral particles suggestive of an adenovirus have been demonstrated in such inclusions by electron microscopy (3). The present authors previously examined turkeys infected experimentally with this disease in which intranuclear inclusion bodies were recognized in reticuloendothelial cells (5). The inclusions spread to most tissues with the exception of the nervous system.

This paper describes the electron microscopic findings in two experimental turkeys which had been subjects in the previous report (5) and attempts to characterize the cells containing the inclusions and the relationship between the affected cells and the viral particles.

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Submitted September 16, 1974.

## MATERIALS AND METHODS

Materials for this investigation consisted of tissues from two experimentally infected turkeys which had been used for a previous report by the present authors and their co-worker (5). One of the birds was infected with a suspension of the spleen and intestines of birds spontaneously affected with hemorrhagic enteritis and killed six days postinoculation. The other was an uninoculated bird exposed to the inoculated bird mentioned above and killed eight days after contact. Histologically both birds showed lesions characteristic of this condition and intranuclear inclusion bodies were found in the spleen, liver, intestines and bone marrow.

Tissues of the spleen, liver, bone marrow and intestines, including the cecal tonsils, were collected from each bird for electron microscopy examination. They were

fixed in glutaraldehyde and 1% buffered osmium tetroxide and embedded in epon<sup>1</sup>. Thin sections were cut and stained with uranyl acetate and lead citrate and then viewed under a Philips 200 electron microscope at 60 KV.

## RESULTS

Intranuclear inclusion bodies were found in all the tissues examined. Cells containing the inclusions were divided into two types, immature cells and reticular cells.

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<sup>1</sup>Fisher Scientific Co., Fairlawn, New Jersey.

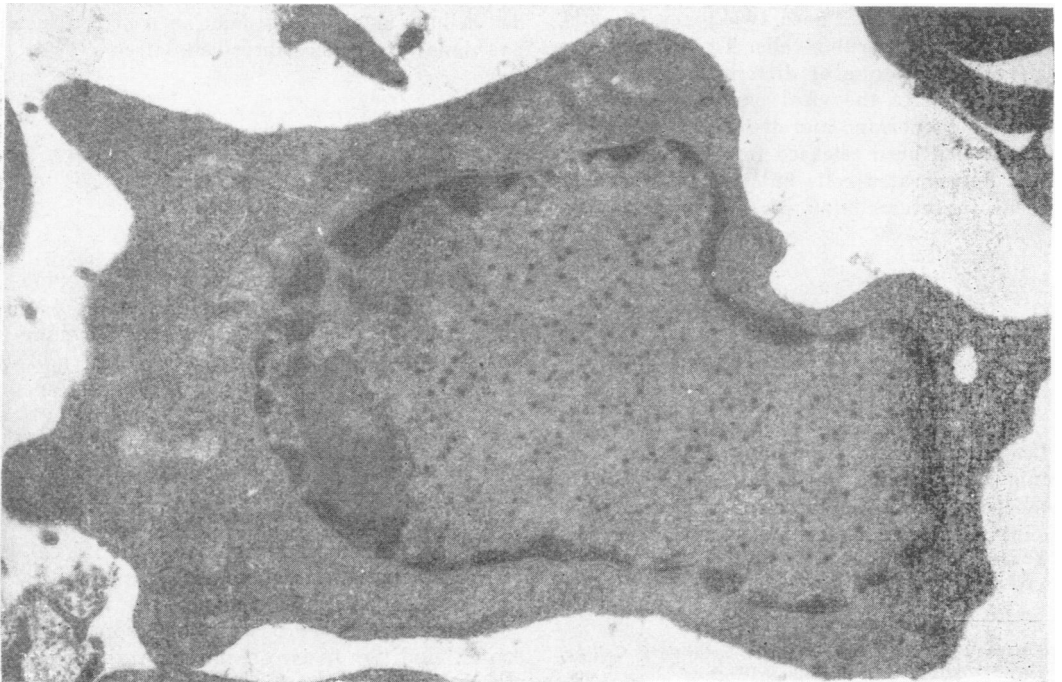


Fig. 1. Spleen. Immature cell. An inclusion body occupies most of the nucleus. The area in which viral particles are diffusely scattered is a little paler than the zone closer to the nuclear membrane. Aggregated chromatin is margined to the nuclear membrane and associating with displacement of the nucleolus. The contour of the cell is amoeboid. The cytoplasm is rich in rough surfaced endoplasmic reticulum, free ribosomes and polysomes with disseminated large mitochondria. X6435.

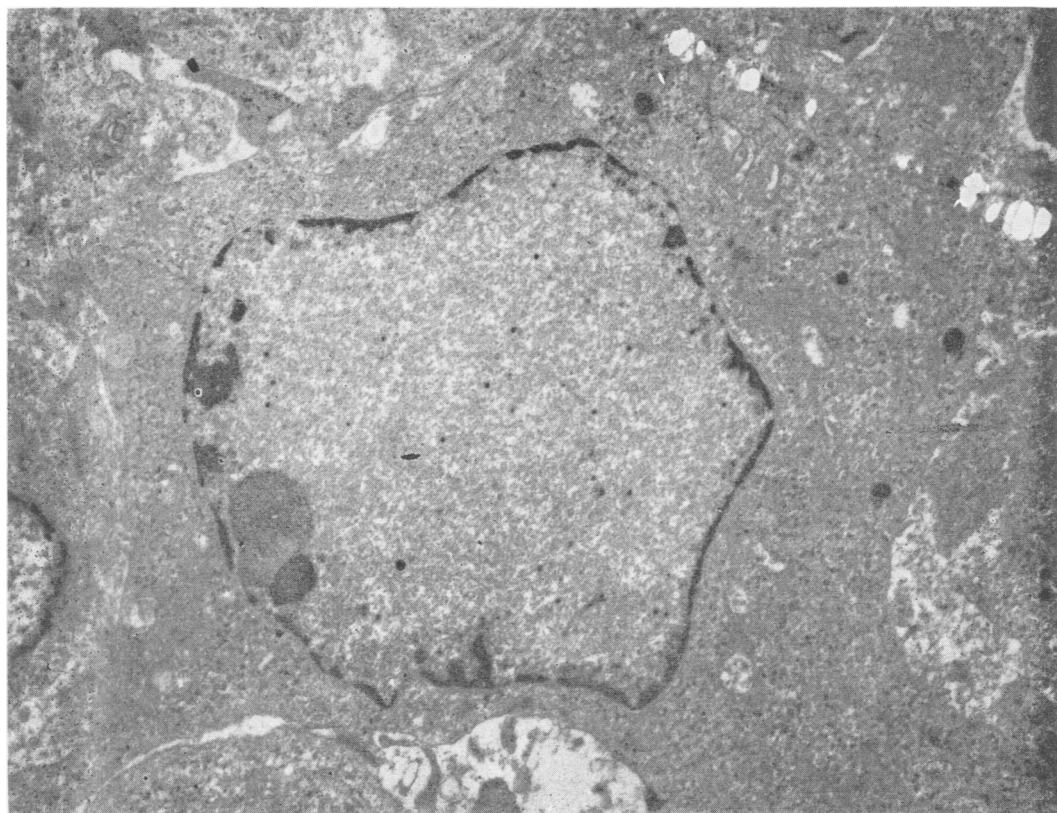


Fig. 2. Spleen. Reticular cell around a sheathed artery. Nuclear changes including an inclusion body are almost identical with those in Fig. 1, except that virus particles are less numerous. The cellular contour is irregular with cytoplasmic processes. In the cytoplasm there are many mitochondria, a moderate number of free ribosomes and polysomes and a few phagosomes and a well developed Golgi apparatus. X6615.

#### IMMATURE CELLS (FIGS. 1 AND 3)

This type of cell occurred in the red pulp of the spleen, sinusoids of the liver, the lamina propria of the intestines, the cecal tonsils and bone marrow. They were rich in cytoplasm, round to irregular and sometimes ameboid in shape and as large as large lymphocytes or free macrophages. In the cytoplasm a large number of free ribosomes and polysomes were present and there were a few to a moderate number of scattered mitochondria. Sometimes a fairly well developed Golgi apparatus was observed. The cells in the spleen and intestines including the cecal tonsils were abundant in rough surfaced endoplasmic reticulum (Fig. 1). On the other hand there was very little endoplasmic reticulum in the cells of the liver and bone marrow. The nuclei were irregular in shape and large in size. The inclusions, with diffusely scattered

viral particles, occupied most of the nucleus. The chromatin was aggregated and adherent to the nuclear membrane (Fig. 3).

#### RETICULAR CELLS (FIGS. 2 AND 4)

This type of cell occurred mainly around sheathed arteries of the spleen. The cells (Fig. 2) were markedly large in size, some as large as free macrophages. They were rich in cytoplasm and irregularly shaped with several cytoplasmic processes. In the cytoplasm a moderate number of mitochondria were scattered and a few phagosomes and a well developed Golgi apparatus were present. The appearance of both the nuclei and the inclusion bodies were almost identical with those in the immature cells except that there were fewer viral particles.

The reticular cells in the bone marrow (Fig. 4) were small in size and spindly in shape. The structures in their cytoplasm

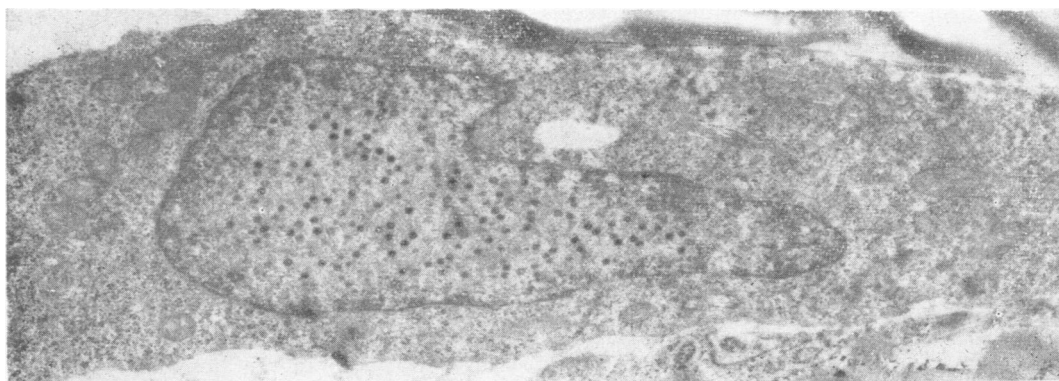
were quite similar to those seen in the reticular cells of the spleen but they had no phagosomes. The inclusions showed the same structure as those in the immature cells. The chromatin was slightly margined at the nuclear membrane but did not aggregate. Nucleoli were not seen.

#### DEGENERATION OF CELLS WITH INCLUSION BODIES (FIG. 5)

In addition to the two types of cells mentioned above, cells with viral particles showed varying degrees of degeneration in the red pulp of the spleen and the bone mar-



**Fig. 3.** Bone marrow. Immature cell. The contour of both cell body and nucleus is irregular. Structures of an inclusion body with dispersed virus particles and lesions of the nucleus are quite similar to those in Fig. 1. The cytoplasm is rich in free ribosomes and polysomes with a few mitochondria. X12,560.



**Fig. 4.** Bone marrow. Reticular cell. This cell is spindle-shaped. An inclusion body identical with that in Fig. 1 occupies most of the nucleus in which there is slight margination of chromatin. The cytoplasm is fairly rich in free ribosomes and polysomes with a moderately large number of mitochondria and a well developed Golgi apparatus. X8545.

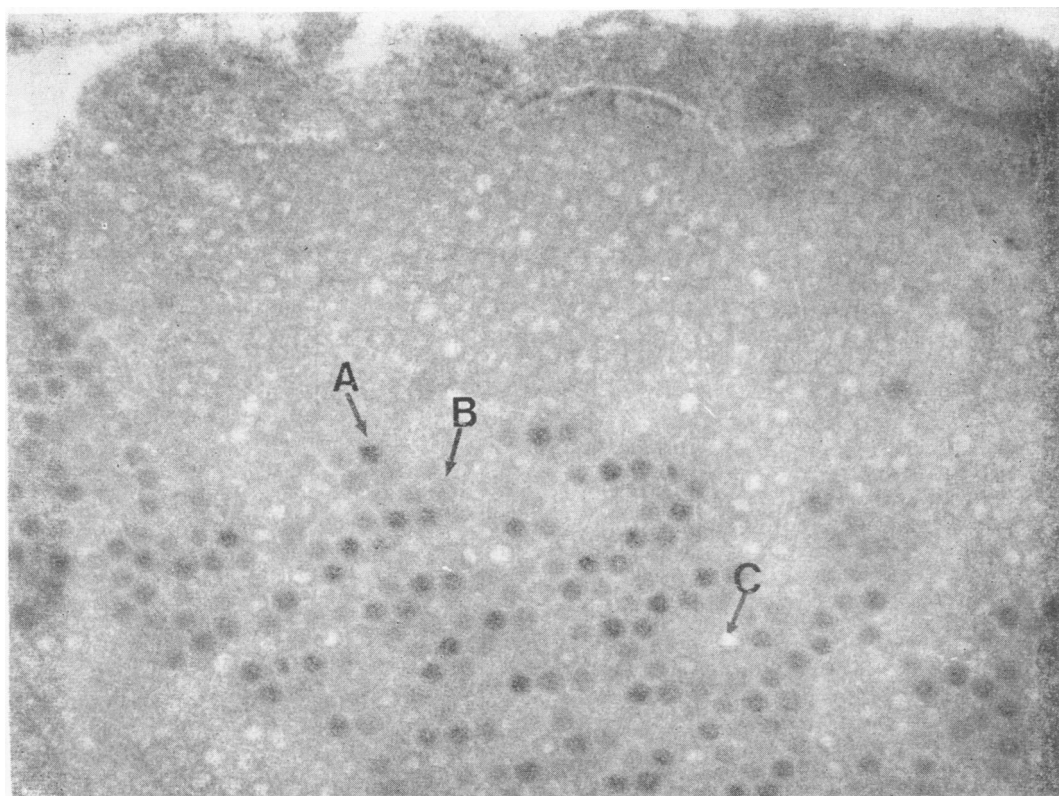


Fig. 5. Bone marrow. Degenerated cell with an inclusion body. A part of the disrupted nuclear membrane and margined chromatin is seen traversing the upper portion of the photomicrograph. The upper part of this area is cytoplasm and the lower, nucleus. The nucleus is filled with three forms of viral particles, particles with a dense osmiophilic core (A), particles containing loose osmiophilic core (B) and particles consisting only of capsids without a core (C). X27,545.

row. They had lost their normal structure to the point that the type of cell could not be identified. However, there were frequently observed aggregates of chromatin and pieces of disrupted nuclear membrane in such cells. Whereas these cells were degenerated, structures of the inclusions themselves were retained fairly well. Viral particles within the inclusions varied in number, ranging from scattered to numerous with a lamellar arrangement (Fig. 5). Occasionally these degenerated cells were surrounded or engulfed by macrophages.

#### INCLUSION BODIES AND VIRAL PARTICLES

The inclusion bodies usually contained viral particles. The area of the inclusions was more granular and somewhat lower in electron density than margined chromatin granules (Figs. 1-4). The viral parti-

cles were divided roughly into three forms according to the electron density of the core although there were various transitional forms between them: (A) particles with a dense osmiophilic core, (B) particles containing a loose osmiophilic core and (C) particles consisting only of capsid without the core (Fig. 5). The particles were approximately 70 nanometers in diameter and round to hexagonal in shape.

Generally, the viral particles of forms A and B were diffusely scattered in the nuclei of cells with intact cytoplasm and nuclear membranes (Figs. 1-4). In the degenerated cells on the other hand the particles tended to be numerous and filled the affected nuclei. These particles were composed of all three forms, A, B and C (Fig. 5) and frequently showed a crystalline arrangement.

Viral particles were not observed in the intercellular spaces.

## DISCUSSION

Two types of cells, immature and reticular, were observed in this investigation. The reticular cells seen in both spleen and bone marrow were not difficult to identify. However, it was not easy to classify the immature cells because of their lack of differentiation. Their characteristic features especially in the bone marrow were the large size, irregular shape, prominent nucleolus, abundance of ribosomes and scantiness of endoplasmic reticulum and mitochondria. Generally speaking it is said that immature cells showing marked proliferation are rich in free ribosomes and poor in endoplasmic reticulum. In addition, Pease (7) has suggested that endoplasmic reticulum increases as the hemocytoblasts transform into myelocytes. The immature cells with well developed rough surfaced endoplasmic reticulum in the spleen and intestines examined in this experiment may be regarded as ones that had differentiated to some degree.

As seen in the light microscopic findings (5) the cells containing the inclusions occurred in tissue areas of the reticuloendothelial system. Accordingly, it is possible that the immature cells described here may belong to the reticuloendothelial system. The virus in this disease may have affected primitive reticular cells.

The inclusions were usually large and contained viral particles. These particles appeared to develop and mature within nuclei. As they increased in number the af-

fected nuclei and cytoplasm were destroyed and a few viral particles were released into the cytoplasm. In addition to the size, shape and arrangement of the particles, their findings suggest some characteristics of adenovirus as described by other workers (1, 2, 4, 6).

In general the progeny particles of DNA viruses tend to be retained in the cells even after viral cytopathic effects have occurred (4). In adenovirus there is no massive release of virus by lysis of the cell (6). These concepts were also evidenced in the present study. There were degenerated cells with viral particles and there was no evidence of viral particles in the intercellular spaces.

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